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PRINCIPAL INVESTIGATOR: Dr. Marisa Buchakjian

CONTRACTING ORGANIZATION: Duke University
Durham, NC 27706

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Dr. Marisa Buchakjian

E-Mail: sally.kornbluth@duke.edu

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14. ABSTRACT

A major obstacle impeding the use of cytotoxic agents in breast cancer treatment is the cancer cell's ability to evade cell death pathways. Therapies which induce apoptosis in otherwise resistant cancer cells have the potential to treat a variety of cancers, including breast cancer. Our research focuses on the apical apoptotic enzyme caspase 2 (C2), which has been proposed as a critical activating protease for breast cancer chemotherapeutic agents such as doxorubicin and etoposide, as well as other genotoxic agents. Our lab has previously shown that C2 is held inactive by nutrient flux through the pentose phosphate pathway (PPP). C2 inactivation in response to nutrient abundance is a consequence of C2 phosphorylation by CaMKII, which prevents downstream engagement of the executioner pathway. CaMKII is itself activated by the PPP byproduct NADPH. We will determine the mechanism of CaMKII activation and subsequent C2 inhibition by NADPH is still unknown. Additionally, we will determine whether via metabolic profiling whether breast cancer metabolism confers resistance to apoptosis.

15. SUBJECT TERMS
None provided.

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Table of Contents

	Page
Introduction……..…..	**2**
Body……..	**3-10**
Key Research Accomplishments……..	**11**
Reportable Outcomes……	**12**
Conclusion……	**13**
References……	**14**
Appendices……	**NA**

INTRODUCTION

Following treatment with chemotherapeutic agents, responsive breast cancer cells undergo apoptotic cell death. This cell death is dependent upon release of cytochrome c from the intermembrane space of the mitochondria to the cytoplasm, where it nucleates a proteolytic machine known as the apoptosome to cleave multiple cellular substrates. The events upstream of the mitochondria that trigger cytochrome c release are not fully understood and unfortunately, breast cancers frequently develop cross-resistance to multiple therapeutic agents, at least in part, by impeding apoptotic progression.

Several groups have shown that the apoptotic protease, caspase-2 (C2), is an essential activator of mitochondrial cytochrome c release in breast cancer cells treated with chemotherapeutic agents such as etoposide. We have found, by knock-down of C2 in breast cancer cells, that C2 is in fact required for responsiveness to etoposide. Work from our laboratory has demonstrated that in *Xenopus* egg extract C2 is normally controlled by the metabolic status of the cell in that high levels of flux through the pentose phosphate pathway (PPP) prevents activation of C2. This inhibition is exerted through phosphorylation of C2 at a specific residue and is catalyzed by the activity of calcium-calmodulin-dependent kinase II (CaMKII). Furthermore, we have determined that CaMKII is activated by a product of the PPP, NADPH, through its ability to support biosynthesis in the *Xenopus* oocyte. The precise mechanism by which NADPH activates CaMKII is an exciting question and is not yet known.

Because breast cancers are known to exhibit increased glucose uptake and biosynthesis**,** we hypothesize that susceptibility of breast cancers to chemotherapeutic agents such as etoposide, reflect, at least in part, the metabolic status of the cells and, consequently, the phosphorylation state of caspase-2. Moreover, because breast cancer cells may exploit elevations in metabolism to evade cell death induced by cytotoxic agents, it is our objective to determine whether caspase-2 is indeed phosphorylated in response to metabolism in these cells, and if so, whether interference with caspase-2 phosphorylation (via inhibition of PPP operation or inhibition of CaMKII activity itself) will sensitize breast cancers to a range of chemotherapeutic agents, thus extending the possibilities for clinical chemotherapeutic combinations.

BODY

Apoptosis is a form of programmed cell death that is activated by various pro-death signals such as DNA damage and oxidative stress[1]. Specifically, apoptosis is characterized by rapid and orderly cellular fragmentation followed by elimination of the apoptotic bodies by phagocytosis. Under normal physiologic conditions, apoptosis of damaged or unneeded cells is precisely balanced by cellular regeneration, thus maintaining appropriate tissue homeostasis. However, disruption of the apoptotic program may manifest as either excessive cell death or inappropriate cell survival. An important example of apoptosis in disease is the development and propagation of cancer cells, which survive despite the presence of prodeath signals from chemotherapeutics, hypoxia, and DNA damage[2]. Cancer cells have been widely described to evade apoptosis by a variety of molecular mechanisms, therefore making them an ideal model for studying the role of apoptosis in pathophysiology.

The apoptotic pathway is coordinated by the activation of a group of cysteine proteases known as caspases, which cleave substrates C-terminal to aspartate residues[3]. My research focuses on the role of the apical apoptotic protein caspase-2 (C2), which is the most evolutionarily conserved caspase and is similar in sequence to other well-described initiator caspases. C2 activity is initiated via induced proximity oligomerization followed by autocatalytic processing for maximal enzymatic activity[3, 4]. Active C2 has been demonstrated to signal through the mitochondrial-dependent pathway of programmed cell death upstream of cytochrome *c* (cyt c) release, and accumulating evidence suggests that C2 is not universally engaged by apoptotic signaling pathways but instead responds to specific cellular stressors such as DNA damage and cytoskeletal disruption following chemotherapeutic treatment in cancer cells[5]. C2 has most commonly been shown to be activated in a complex containing the adaptor protein RAIDD and death domain-containing protein PIDD, however, additional adaptor proteins have been identified and, interestingly, cells from a PIDD-/- mouse show no defects in C2 signaling[6, 7]. Once active, C2 cleaves its substrates including a pro-apoptotic member of the Bcl-2 family (Bid), which, when cleaved, promotes Bax translocation to the mitochondria and subsequent cyt *c* release[8].

Despite what is currently known about C2 signaling, progress on the details of this pathway was stalled for several years after characterization of the C2 knockout mouse revealed only a mild phenotype, including excess oocytes[9]. Of interest, C2 knockout oocytes were also determined to be markedly chemoresistant compared to their wild-type counterparts, suggesting a role for C2 in both normal and pathophysiological processes[9]. The results of the C2 knockout studies were of particular interest to our lab where we study cell death pathways using the *Xenopus* egg extract system. It has been known for over a decade that *Xenopus* egg extract, when left to sit at room temperature, will display many of the classical signs of apoptosis including caspase activation and cyt *c* release[10]. However, the initiating factor in *Xenopus* extract apoptosis remained unknown. In a 2005 publication in *Cell*, our lab demonstrated that apoptosis in the *Xenopus* extract system is initiated by the depletion of critical

nutrients over time, which eventually results in activation of the apical enzyme C2[11]. The manuscript established that C2 is held in an inactive state in the presence of flux through the pentose phosphate pathway (PPP) to produce sufficient NADPH, which in turn acts to promote an inhibitory phosphorylation of C2 at serine 135 by CaMKII. This work demonstrates that oocyte nutrient flux is crucial in preventing C2-mediated apoptosis in the *Xenopus* egg extract system, thus defining a mechanism of C2 suppression[11].

Our lab has previously shown that C2 is held inactive by nutrient flux through the pentose phosphate pathway via phosphorylation at serine 135, which is mediated by calmodulin-dependent protein kinase II (CaMKII). We have also reported recent exciting advancements in the understanding of caspase-2 metabolic regulation using *Xenopus laevis* egg extract, which was also detailed in the progress report from 2009[12]. This work demonstrated that caspase-2 phosphorylated at S135 binds 14-3-3ζ, thus preventing caspase-2 dephosphorylation. Moreover, we determined that S135 dephosphorylation is catalyzed by protein phosphatase-1, which directly binds caspase-2. Although caspase-2 dephosphorylation is responsive to metabolism, neither PP1 activity nor binding is metabolically regulated. Rather, release of 14-3-3ζ from caspase-2 is controlled by metabolism and allows for caspase-2 dephosphorylation. Accordingly, a caspase-2 mutant unable to bind 14-3-3ζ is highly susceptible to dephosphorylation.

Although this mechanism was initially established in *Xenopus*, we have also demonstrated similar control of murine caspase-2 by phosphorylation and 14-3-3 binding in mouse eggs[12]. The fact that this mechanism is conserved in mammalian oocytes supports that, in fact, caspase-2 is likely to be metabolically regulated even in the mammalian system. This extensive biochemical work, on which I am a co-first author, was accepted for publication in the June 16, 2009, edition of *Developmental Cell* as a featured article. This work is crucial to our understanding of the role of caspase-2 and metabolism in breast cancer because it extends our biochemical understanding of caspase-2 and its interactions with other proteins. Importantly, a paper was recently published which reports that 14-3-3ζ (the isoform implicated in caspase-2 phospho-S135 binding and suppression) overexpression in breast cancer defines high risk of disease and promotes cancer cell survival[13]. This is extremely interesting given our current findings in that it supports conservation of the mechanism of caspase-2 activation in breast cancer cells.

One goal of our proposal was to determine whether C2 is a metabolically-regulated phosphoprotein in mammalian somatic cells, and if so, to identify the C2 motif phosphorylated in response to the pentose phosephate pathway. As shown in Figure 1, C2 is, in fact, a metabolically-regulated phosphoprotein in cells, with changes in phosphorylation status evident following treatments that impinge upon NADPH levels.

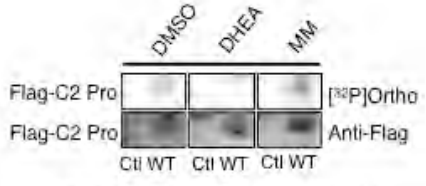

Figure 1. Flag-C2 prodomain was transfected into 293T cells. Cells were treated with DHEA or MM and then phosphate-starved and incubated in the presence of orthophosphate. Flag-C2 prodomain was immunoprecipitated on anti-flag agarose, and the SDS-PAGE gel was exposed by autoradiography.

After determining that C2 is phosphorylated in somatic cells, we set out to characterize the metabolically-regulated phosphorylation site in both mouse and human C2. In examining homology of the mouse and human proteins with *Xenopus* C2, it was evident that a conserved serine (S164) exists that is homologous to *Xenopus* S135. However, previous work from other labs has also identified a nearby residue, S157, as being regulated by phosphorylation in a disparate context[14]. To identify which site might be regulated by metabolism, we generated and affinity-purified mouse C2 antibodies direct against either phospho-S157 or phospho-S164. These antibodies have been validated and are specific for phosphorylation only at the designated epitope. Using these antibodies, we were able to determine that S164 is the metabolically-regulated phosphorylation site on mouse C2 (Figure 2). These data have also been repeated using human C2 constructs, which also demonstrates S164 as the regulated site in mammalian somatic cells.

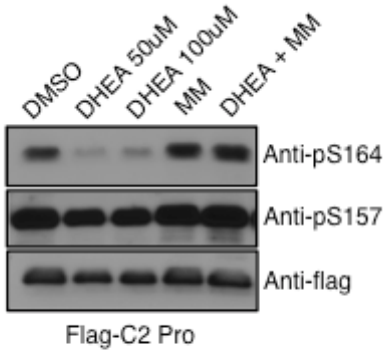

Figure 2. Flag-C2 prodomain was transfected into 293T cells. Cells were starved of glucose and then treated with DHEA or MM. Whole cell lysates were made and immunoblotted for flag, phospho-S157, and phospho-164.

After determining the metabolically-regulated phosphorylation site on C2, we wanted to examine the effects of C2 phosphorylation on its activation. C2 activation is initiated by induced proximity, and optimal activity is then achieved by autocatalytic processing of the procaspase. To examine the effects of C2 phosphorylation on C2 oligomerization and induced proximity, we utilized a novel and exciting technique recently described in the literature that employs bimolecular fluorescence complementation (BiFC)[4]. A recent publication demonstrated single-cell visualization of C2 induced proximity activation using C2 variants fused to split framents of the fluorescent protein Venus. When the BiFC C2 pairs are transiently transfected into HeLa cells, the cells become Venus positive as observed by fluorescence when a C2-activation stimulus is applied (PIDD/RAIDD overexpression, heat shock, etoposide). Using this technique, it is possible to visualize the extent of C2 activation by induced proximity, providing an accurate and sensitive assay for measuring C2 oligomerization. We have obtained the wild-type plasmids for these expression constructs, and have also synthesized C2 phospho-mutant and phosphomimetic variants in order to compare C2 activation in the setting of metabolic treatments. As shown in Figure 3, C2 mutated at S164 is no longer responsive to metabolic regulation when treated with the cytotoxic drug Paclitaxel as compared to WT C2, suggesting a critical role for phosphorylation of this site in preventing C2 activity.

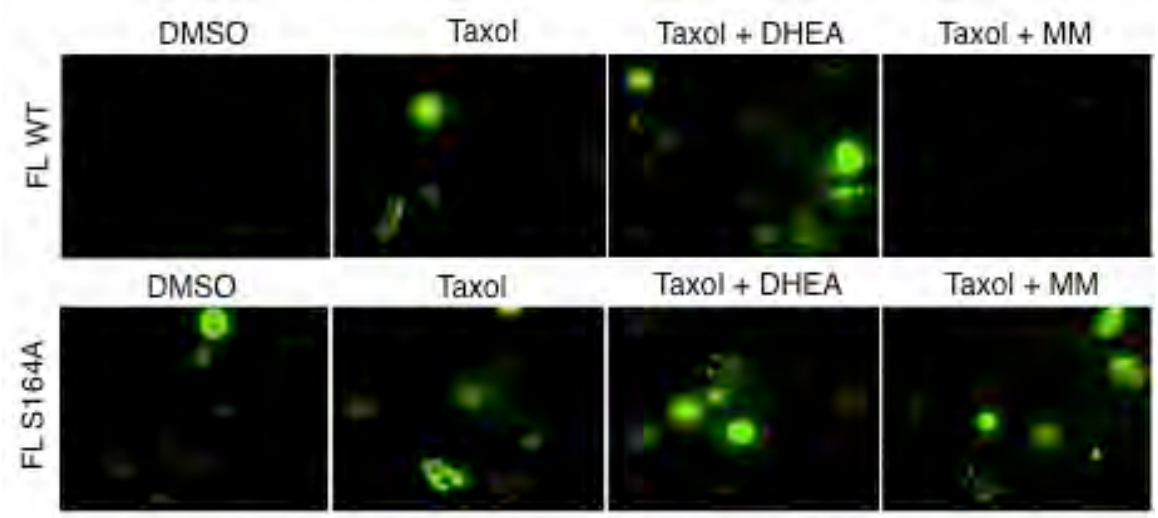

Figure 3. Full-length caspase-2 WT or S164A fused to either the N- or C-terminal half of the Venus fluorescent protein (VFP) were transfected into HeLa cells (MitoRed was also transfected to mark cells expressing exogenous constructs). Cells were treated with paclitaxel, DHEA, and MM and analyzed for C2 activation via confocal microscopy for VFP.

We are also performing studies to determined the C2 S164-directed kinase and phosphatase, and have preliminary data to suggest that, in agreement with the *Xenopus* C2 model, CaMKII and protein phosphatase-1 might be important in dictating the phosphorylation status of mammalian C2 S164.

Our current work is aimed at using the human C2 phospho-S164 antibody to extend the paradigm of metabolic regulation of C2 to a pathophysiologic setting, with a specific focus on the effects of C2 phosphorylation on chemosensitivity in breast cancer. In breast cancer, cytotoxic chemotherapeutics such as etoposide remain at the forefront of treatment, and resistance to these agents is a major obstacle in achieving disease remission and cure. Evasion of apoptosis is one of the hallmarks of cancer, and transformed cells have evolved mechanisms to inhibit cell death pathways even in the presence of pro-death cytotoxic agents[15]. Furthermore, tumor cells also exhibit aberrant metabolic activity characterized by high glucose uptake, thus leading to increased levels of G6P and NADPH[16]. According to our previous studies, high levels of G6P in cancer cells could cause increased flux through the PPP, and, consequently, increased NADPH production and C2 inhibition via S164 phosphorylation. C2 signaling is of particular interest to the study of breast cancer because reports have shown that cytotoxic agents such as etoposide are thought to signal through C2 in order to promote apoptosis[5, 17]. In fact, our lab has determined that knock-down of C2 by RNAi in MDA-MB-231 cells prevents etoposide-induced death (Figure 4). The purpose of this portion of the project is to elucidate the role of C2 phosphorylation in breast cancer, with the goal of better understanding C2 phosphorylation in cancer chemosensitivity. We hypothesize that cancer cell resistance to C2-dependent chemotherapeutics is, in part, mediated by inhibition of C2 via metabolically-regulated phosphorylation.

Our lab has obtained several breast cancer cell lines, and we are in the process of scoring the lines for varying resistance to the commonly used chemotherapeutic agent etoposide. Using the breast cancer cells, we will first confirm that these treatments are dependent on C2 signaling using C2 RNAi and inhibitor studies. If C2 is required for apoptosis, we will then examine total C2 expression as well as C2 phosphorylation status and compare this to etoposide sensitivity. We might expect that the cell lines most resistant to treatment might also display the greatest relative levels of C2 phosphorylation.

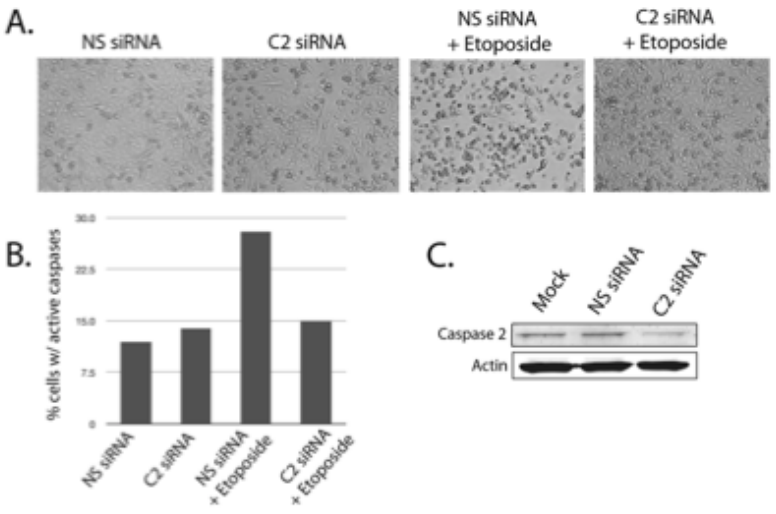

Figure 4. MDA-MB-231 cells were treated with mock or C2 siRNA constructs and then incubated with etoposide. A) Brightfield pictures of cell death in the presence of the various treatments. B) FACS analysis of cleaved caspase-3 in the treated cells. C) Extent of C2 knock-down.

Once we have determined the basal C2 phosphorylation state of each breast cancer cell line, we will examine C2 phosphorlation and chemoresponsiveness in the setting of metabolic manipulations to affect NADPH levels. We would expect to ovserve a syntergistic effect on cell death with inhibition of NADPH production, and protection from death with NADPH augmentation. We would also expect that changes in cell death levels would coordinate with C2 S164 phosphorylation status. It would also be of interest to knock down endogenous C2 and replace it with either siRNA-inert C2 wild-type or phosphomutant to determine whether manipulation of C2 phosphorylation status alone can alter the cell's responsiveness to chemotherapeutics. Germane to this proposal, it would also be of great interest to apply our findings of C2 in breast cancer to a clinical setting, and we have obtained access to primary human tumor samples which will allow us to evaluate the C2 phosphorylation status in disease.

Additional comments on progress from the mentor, Dr Kornbluth (as Dr. Buckakjian has now moved on to her medical residency):

Another goal of Dr. Buchakjian's proposal was to determine the mechanism whereby robust metabolism could activate CaMKII to phosphorylate caspase 2. Our laboratory (with a great deal of contribution by Dr. Buckakjian) made excellent progress on this aim over the course of Dr. Buchakjian's DOD funding.

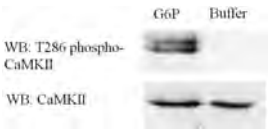

Fig. 5. Egg extract incubated in the presence or absence of G6P was resolved by SDS-PAGE and immunoblotted for phospho-Thr 286 on CaMKII or for total CaMKII.

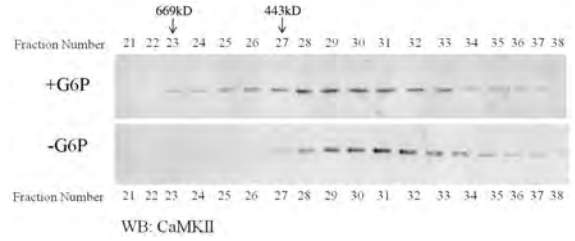

Fig. 6. Egg extracts incubated +/-G6P were separated by gel filtration on a superpose 6 column. Fractions were then immunoblotted with anti-CaMKII antibody.

Dr. Buchakjian, working with another student, Bofu Huang, found that NADPH-induced stimulation of CaMKII in egg extract occurred robustly even in the absence of membrane compartments and Ca^{2+} stores, indicating that it is independent of [Ca^{2+}] elevation. However, Glucose 6 phosphate addition to Xenopus egg extracts did promote phosphorylation of T286, an event dependent on oligomerization (Fig 5). This prompted us to ask whether abundant nutrients might promote CamKII oligomerization. Indeed, CaMKII underwent a marked shift in its gel filtration profile to a larger size upon addition of G6P; identical results were obtained when the caspase inhibitor zVAD-fmk was included in during the extract incubation (Fig 6). Although this could, in theory, be due to either formation of the oligomerized holoenzyme or recruitment of additional proteins to the CaMKII complex, it is clear that the specific activity of the higher molecular weight fractions was significantly elevated. Thus, metabolism might regulate CaMKII via a novel mechanism involving induced oligomerization or novel protein complex formation.

To determine whether post-translational modification of CaMKII might result in altered CaMKII oligomerization/complex formation, Dr. Buchakjian and Mr. Huang retrieved CaMKII from egg cytosol treated +/- G6P using CaM sepharose and analyzed the protein by Mass Spectrometry (in collaboration with the Duke Proteomics Facility). Aside from elevated autophosphorylation (expected to parallel kinase activity), the most notable change upon G6P treatment was a >100-fold decrease in phosphorylation of CaMKII T393 and S395 (Xenopus g isoform L subunit numbering, equivalent to T371/S373 in the human homolog), which lie directly within the oligomerization domain. These observations, coupled with the fact that we have not observed any G6P-induced novel CaMKII interactors by either Mass Spectrometry or SDS-PAGE/silver staining following G6P treatment, prompt us to hypothesize that phosphorylation of T393/S395 impedes proper CaMKII oligomerization of the holoenzyme, and that their dephosphorylation in nutrient replete conditions alleviates this inhibition to allow CaMKII activation. Based on studies in nutrient-replete conditions, CaMKII is often considered a constitutive oligomer, but we

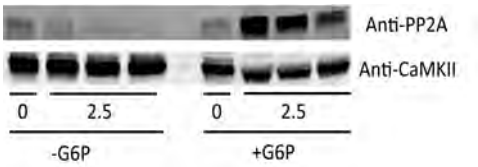

Fig. 7. Calmodulin Sepharose was incubated in crude egg extracts followed by treatment of the extracts with or without G6P. Samples were taken at 0 and 2.5 hours following treatment and binding of the PP2A catalytic subunit to CaMKII was assessed by immunoblot. The 2.5 hour time point shows samples washed with buffers of increasing stringency (0mM NaCl, 300mM NaCl, and 300mM NaCl +0.1% NP40, respectively). Note that similar results were obtained with anti-CaMKII immunoprecipitations followed by PP2A immunoblotting.

suspect that CaMKII status may never have been examined following nutrient depletion (as in the egg extract after nutrient exhaustion). G6P treatment promoted binding of PP2A (but not PP1) to CaMKII (Fig. 7 and data now shown), and okadaic acid treatment at concentrations that inhibit PP2A largely abrogated the G6P-induced change in the CaMKII gel filtration profile (Fig. 8). These data suggest that metabolic control of CaMKII may involve T393 and S395 dephosphorylation by PP2A.

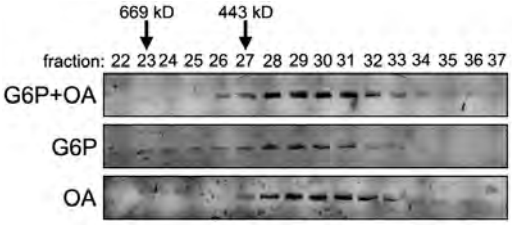

Fig. 8. Egg extracts were treated +/- G6P in the presence or absence of the phosphatase inhibitor okadaic acid. Extracts were separated by gel filtration and immunoblotted for CaMKII.

Statement of Work

A. Training plan tasks

1. Attend weekly Signal Transduction Colloquia sponsored by the Department of Pharmacology and Cancer Biology, interact with seminar speakers
   Attended all appropriate seminars
2. Attend weekly Science of Oncology Symposia sponsored by the Duke Comprehensive Cancer Center
   Attended all Science of Oncology seminars
3. Attend oncology clinic at the Duke Breast Program
   Attended breast oncology clinic twice
4. Develop skills to effectively ask scientific questions
   Achieved
5. Practice thoughtful, creative, and effective experimental design
   Achieved
6. Analyze the implications for public health and human disease in all of my scientific work
   Achieved
7. Develop effective scientific writing techniques by preparing manuscripts
   Wrote both a Developmental Cell paper and a review article for Nature Reviews in Molecular and Cellular Biology
8. Maintain a strong fund of knowledge of the published literature in breast cancer and apoptosis
   Achieved

Research tasks and Methods

1. Determine the mechanism of CaMKII activation by NADPH
   a. CaM/CaMKII binding assays in the presence and absence of G6P/NADPH in *Xenopus* egg extract
   Discovered differential binding of PP2A to CamKII in the presence and absence of G6P/NADPH.

b. Recombinant glutathione-bound CaMKII in the presence and absence of G6P/NADPH in *Xenopus* egg extract

i. Silver staining

ii. 2D gel electrophoresis

iii. Mass spectrometry to identify novel binding partners

Produced GST-CaMKII as well as the hypophosphorylated mutant variant (T393, S395A).

c. NADPH affinity binding column for depletion of *Xenopus* egg extract of NADPH-interacting factors

i. Evaluate depleted fractions for C2 phosphorylation activity in the presence of exogenous NADPH

ii. Elution of NADPH binding partners and add-back of eluate to depleted extract to test for recovery of C2 phosphorylation activity

We took an alterantive approach as we discovered (work of another postdoctoral fellow in the laboratory that NADPH requirement was as a precursor for fatty acid synthesis. In other words, the effects of NADPH were not due to direct binding of NADPH to an effector.

d. Evaluate CaMKII activity in presence and absence of G6P/NADPH

i. Immunoblotting with phospho-T286 CaMKII antibody

ii. *In vitro* kinase assays to look at CaMKII phosphorylation at novel sites, mapping of novel sites

As shown above, we found that CaMKII at its autophosphorylation site (T286) is increased. We also found decreased phosphorylation at two sites on CaMKII, which we believe are responsible for promoting CaMKII activation in response to NADPH-stimulated fatty acid synthesis.

2. Analysis of C2 phosphorylation status in normal and breast cancer cell lines

a. Use a phospho-C2 antibody to analyze various breast cancer cell lines

i. Production of phospho-C2 antibody

ii. Immunoblotting a spectrum of breast cancer cell lines with phospho-C2 antibody

Status: We have made phospho-antibodies directed against mouse C2 S157 and S164, and human C2 S164. We have demonstrated that these antibodies are specific both for the epitope as well as the modification. Using these antibodies we have been able to determine that S164 phosphorylation status is regulated by metabolism. We are currently using these antibodies to evaluate breast cancer cell lines, with the hope of eventually moving these studies into primary tissue samples to understand the role of C2 phosphorylation in cancer chemosensitivity.

iii. Extend analysis to primary tumor samples and correlate C2 phosphorylation status with disease progression

We have now managed to optimize use of the phosphoantibodies for immunostaining and are poised to stain tumor samples.

b. Introduction of C2 phospho-mutants to chemoresistant cells

i. Treat with chemotherapeutics such as doxorubicin and etoposide, in the presence and absence of DHEA and/or malate

ii. Determine chemosensitivity using a cleaved caspase-3 antibody

Status: Currently optimizing cell line treatment conditions, and will evaluate the cell lysates with the C2 phosho-antibody as well as antibodies to demonstrate apoptosis.

3. Analysis of CaMKII activity in normal and breast cancer cell lines
    a. Evaluate cell lines using an autophosphorylated-CaMKII antibody (phospho-T286)
    b. Evaluate activity of cell lysates against a radioactive CaMKII substrate
    c. Extend analysis to primary tumor samples and correlate CaMKII activity with disease progression

We screened some breast cancer cell lysates by immunoblotting with the pT286 CaMKII antibody, and see some appreciable differences in CaMKII activity using the canonical autophosphorylation site as a readout. We are now correlating this with phospho-caspase 2 staining.

4. Metabolomic profiling of breast cancer cell lines
    a. Use mass spectrometry to measure intermediary metabolites
    b. Use statistical analysis to create metabolic "signatures" for each cell line
        i. Create a signature to differentiate between normal and cancerous breast epithelial cells
        ii. Correlate metabolomic data with analysis of CaMKII activity and C2 phosphorylation status

Status: Once breast cancer cell lines with differing CaMKII activity and C2 phosphorylation status have been identified, we will use these cell lines to pursue metabolic profiling to create "signatures" for each selected cell line.

RESEARCH ACCOMPLISHMENTS of Dr. Buchakjian

- Caspase-2 is dephosphorylated at S135 in *Xenopus laevis* egg extract prior to caspase-2 processing and downstream effector caspase activation
- Caspase-2 dephosphorylation is inhibited in the presence of G6P to stimulate the pentose phosphate pathway
- Inhibition of PP1 by okadaic acid addition prevents caspase-2 dephosphorylation, caspase-2 processing, cytochrome *c* release, and downstream caspase activation
- PP1 depletion using GST-Inhibitor-2 prevents caspase-2 dephosphorylation
- PP1 directly binds caspase-2 in the absence of a targeting subunit
- PP1 binds caspase-2 at V106/H108
- PP1 binding and activity are not regulated by metabolism
- Caspase-2 phosphorylated at S135 selectively binds 14-3-3ζ but not other 14-3-3 isoforms
- 14-3-3 release from caspase-2 occurs immediately prior to maximal caspase-2 dephosphorylation and downstream caspase activation
- 14-3-3 release is inhibited in the presence of G6P, and 14-3-3 binding is the point of metabolic control in caspase-2 activation
- The mechanism of caspase-2 activation is conserved in mouse caspase-2 in oocytes
- Mammalian caspase-2 phosphorylation is metabolically-regulated in somatic cells
- Mammalian caspase-2 is phosphorylated at S164 in a metabolically-sensitive manner in somatic cells
- Caspase-2 is phosphorylated by CaMKII and dephosphorylated in PP1 in somatic cells, in a manner analogous to the *Xenopus* mechanism
- Caspase-2 phosphorylation at S164 in mammalian somatic cells prevents induced proximity activation
- PP2A binds to CaMKII in a nutrient-regulated manner
- Nutrients alter the sedimentation of CaMKII, potentially indicating altered oligomerization status
- Nutrients alter phosphorylation of CaMKII at its autophosphorylation site, indicative of enzymatic activation.

REPORTABLE OUTCOMES

The engine driving the ship: metabolic steering of cell proliferation and death.

Buchakjian MR, **Kornbluth S**.

Nat Rev Mol Cell Biol. 2010 Oct;11(10):715-27..

PMID: 20861880

Restraint of apoptosis during mitosis through interdomain phosphorylation of caspase-2.

Andersen JL, Johnson CE, Freel CD, Parrish AB, Day JL, **Buchakjian** MR, Nutt LK, Thompson JW, Moseley MA, **Kornbluth S**.

EMBO J. 2009 Oct 21;28(20):3216-27.

PMID: 19730412

Metabolic control of oocyte apoptosis mediated by 14-3-3zeta-regulated dephosphorylation of caspase-2.

Nutt LK, **Buchakjian** MR, Gan E, Darbandi R, Yoon SY, Wu JQ, Miyamoto YJ, Gibbons JA, Andersen JL, Freel CD, Tang W, He C, Kurokawa M, Wang Y, Margolis SS, Fissore RA, **Kornbluth S**.

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CONCLUSIONS

Our lab has previously shown that C2 is held inactive by nutrient flux through the pentose phosphate pathway via phosphorylation at serine 135, which is mediated by calmodulin-dependent protein kinase II (CaMKII). During the course of this grant, we made exciting advancements in the understanding of caspase-2 metabolic regulation using *Xenopus laevis* egg extract and in mammalian somatic cells. We have determined that caspase-2 phosphorylated at S135 binds 14-3-3ζ, thus preventing caspase-2 dephosphorylation. Moreover, we determined that S135 dephosphorylation is catalyzed by protein phosphatase-1, which directly binds caspase-2. Although caspase-2 dephosphorylation is responsive to metabolism, neither PP1 activity nor binding is metabolically regulated. Rather, release of 14-3-3ζ from caspase-2 is controlled by metabolism and allows for caspase-2 dephosphorylation. Although this mechanism was initially established in *Xenopus*, we now demonstrate similar control of murine caspase-2 by phosphorylation and 14-3-3 binding in mouse eggs. We also demonstrate that both mouse and human caspase-2 are metabolically-regulated phospho-proteins in mammalian somatic cells. We have further identified the regulated phosphorylation site in somatic cells as S164, and are currently working toward understanding the effects of C2 phosphorylation at S164 on chemoresponsiveness in breast cancer cells. Finally, we have made inroads into the mechanism by which CaMKII is controlled by metabolism, implicating nutrient-regulated PP2A-CaMKII interactions in controlling CaMKII oligomerization/activation status.

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